

# Lower urinary pH is useful for predicting renovascular disorder onset in patients with diabetes

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## ABSTRACT

**Background and objectives:** A lower urinary pH (UpH) is closely linked to diabetes. However, its relation to diabetic renovascular damage is unclear. This study aimed to identify the relationship between UpH and the exacerbation of diabetic renovascular disorders.

**Methods:** This is a 10-year observational study targeting 400 outpatients with diabetes who registered in 2003. We investigated the relationship between UpH in 2003 and renovascular damage from 2003 to 2013.

**Results:** A total of 350 participants were eligible for the analysis. During their 10-year outpatient treatment, a decrease was seen in glycated hemoglobin levels, blood pressure, and estimated glomerular filtration rates (eGFRs), and an increase was seen in their urinary albumin–creatinine ratios (ACRs), uric acid (UA) levels, and intima-media thickness (IMT). UpH negatively correlated with urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), body mass index, UA, and ACR, and positively correlated with eGFR. The results of a multiple regression analysis showed that the independent risk factors for UpH were 8-OHdG, UA, eGFR, and ACR. UpH also negatively correlated with the percent change in IMT (%IMT), the percent change in pulse wave velocity (%PWV), and the change in log ACR ( $\Delta$ log ACR), and positively correlated with the percent change in eGFR. A multiple regression analysis revealed that UpH was an independent risk factor for the %IMT, %PWV and  $\Delta$ log ACR. Obese patients with low UpH values frequently suffered from sleep apnea syndrome.

**Conclusions:** These results suggest that UpH is a useful marker for predicting the onset of renovascular disorder in patients with diabetes.

## INTRODUCTION

Lower urinary pH (UpH), which is common among complicated case patients such as obesity and chronic kidney disease (CKD), contributes to precipitation of uric acid (UA) in the urine.<sup>1</sup> For UA to precipitate in the urine, either the urinary concentration of UA must rise past its saturation point or the UpH must drop. The serum UA concentration is elevated in patients with metabolic syndrome.<sup>2</sup> Low UpH is closely linked to the

## Key messages

- A lower urinary pH is closely linked to diabetes, as well as to the exacerbation of diabetic renovascular disorders.
- A lower urinary pH is closely linked to the metabolic syndrome and hyperuricemia, and also strongly related to increases in intrarenal oxidative stress.
- The mechanism that lower urinary pH takes part in the progress of diabetic renovascular disorders has not been clarified.

accumulation of visceral fat, insulin resistance (IR), methylglyoxal concentration, and hypertension.<sup>1</sup> It is also strongly related to increases in intrarenal oxidative stress (OS) and the acceleration of the renin-angiotensin system (RAS).<sup>1</sup>

A decrease in UpH occurs because of an increased supply of hydrogen ions ( $H^+$ ) to the urine, or decreased elimination of urinary  $H^+$ . The former occurs because of increased blood  $H^+$  (acidosis) or increased  $H^+$  secretion from the renal tubules to the urine, whereas the latter occurs because of a reduction in the supply of ammonium ( $NH_3$ ) to the urine. Acidosis takes various forms, such as respiratory acidosis and metabolic acidosis. In diabetics, decreased urinary  $NH_3$  contributes to a drop in UpH.<sup>3</sup>

Researchers have reported that a decrease in UpH is a predictive factor for CKD,<sup>4</sup> that a lower UpH reinforces OS inside the renal tubules via albumin reabsorption, mediated by the reinforcement of proline-rich tyrosine kinase 2,<sup>5</sup> and that when UpH is elevated via the administration of sodium bicarbonate, the incidence of initiation of dialysis in CKD is reduced.<sup>6</sup> These reports strongly suggest that a lower UpH is closely linked to the advancement of renovascular damage. However, there are very few studies in patients with diabetes that examine the relationship between lower UpH and the advancement of renovascular damage. We

therefore examined the influence of lowered UpH on the 10-year progression of renovascular disorders.

## METHODS

This is a prospective observational cohort study targeting 400 patients with diabetes who registered between January and March 2003. We finalized the baseline data (taken at the start of the observation) using the values obtained at the clinical examinations performed at the time of registration between January and March 2003 (collection of first morning urine and spot blood draw), as well as the values of the clinical tests performed once every 3 or 4 months during a 1-year period from April 2003 to March 2004 (spot urine and spot blood draw). For urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG: an OS marker), waist circumference (WC), pulse wave velocity (PWV), and intima-media thickness (IMT), the values measured at the time of registration in 2003 were employed as the baseline values. For data other than these, the average value of the measurements taken at the time of registration and the three measurements obtained once every 3–4 months within the subsequent year (a total of 4 measurement values, including the data at the time of registration) were used as the baseline (2003–2004) data. This process was used to minimize deviations in the UpH values, which are liable to fluctuate and are influenced by meals and exercise, and in body weight and estimated glomerular filtration rate (eGFR), which show large seasonal fluctuations. Likewise, the average value of the first morning urine collection and spot blood draw, taken just once between January 2012 and March 2013, and the three data measurements obtained at an interval of 3–4 months within the subsequent year (spot urine collection and spot blood draw: a total of 4 measurement values) were used as the 2013 end point data. WC, PWV, and IMT were measured just once at the end point between January 2012 and March 2013. The items measured during regular medical examinations included body mass index (BMI), blood pressure (BP), heart rate, a urine test, liver function, renal function, UA, a peripheral blood test, casual blood glucose (BG), glycated hemoglobin (HbA1c), serum lipids, electrolytes, and the urinary albumin–creatinine ratio (ACR). The details are shown in [table 1](#). The treatment with drugs believed to affect renovascular disorders and the changes in the administration of these drugs between March 2004 and March 2013 were investigated and are shown in [table 2](#).

UpH was measured using an AX-4030 (ARKRAY, Inc, Kyoto, Japan), a fully automated urine analyzer with a preset test paper. The first morning urine was collected after a fasting period of more than 12 h. To minimize the influence of meals, exercise, seasonal fluctuations, etc, we averaged the four UpH values collected every 3 months during the course of a year and designated it as the UpH for that particular year. We asked the participants to restrict themselves to either a diabetic diet or a

renal diet that complied with various instructions 48–72 h before examination, and prohibited them from engaging in strenuous physical exercise.

Sleep apnea syndrome (SAS) was diagnosed as appropriate based on patient reports of night-time apnea, snoring, daytime somnolence, and dissatisfaction with sleep, and the participants were referred to specialists as needed. Fatty liver was diagnosed by specialists using abdominal echo and/or CT.

This study complied with the Helsinki Declaration and was conducted with the approval of the Medical Ethics Committee of Tohoku University. All participants provided their full informed consent.

## STATISTICAL ANALYSIS

We are planning a study of a continuous response variable from independent control and experimental participants with 1 control(s) per experimental participant. In a previous study, the response within each participant group was normally distributed with an SD of 20. If the true difference in the experimental and control means is 6, we will need to study 175 experimental participants and 175 control participants to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with a probability (power) of 0.8. The type I error probability associated with this test of this null hypothesis is 0.05.

Numerical values that were normally distributed are presented as the mean±SD, and those that were not normally distributed are presented as the median (range). To compare the values at baseline and end point, Student t test was used for data sets that had a normal distribution and the Wilcoxon signed-rank test was used for those that did not. The  $\chi^2$  test was used to compare the rates of drug administration, and the Spearman test was used to study correlations. Furthermore, a multiple regression analysis was performed with UpH as the dependent variable and the items that were individually correlated with UpH as the independent variables. Additional multiple regression analyses were performed that used the change in log ACR ( $\Delta\log$  ACR), the percent change in eGFR (%eGFR), the percent change in PWV (%PWV), and the percent change in IMT (%IMT) between the baseline and end point each as dependent variables, and the independent variables individually correlated with the respective dependent variables. For all tests,  $p < 0.05$  was regarded as significant.

## RESULTS

During the 10-year period, 50 patients dropped out of the study ( $n=350$ ). Seven died; 15 were transferred to other institutions; 2 withdrew their consent to be enrolled in the study; 16 went missing; and 10 had missing data. This large number of dropout cases is likely to have been due to the Great East Japan Earthquake of 2011.

**Table 1** The study participants' demographic and clinical data at the baseline and end point

	Baseline (2003–2004)	End point (2012–2013)	p Value
Age (years)	53.7±13.6	–	–
Gender (M/F)	157/193	–	–
Duration (years)	11.8±7.8	–	–
BMI (kg/m <sup>2</sup> )	25.5±5.0	25.5±5.0	0.802
WC (cm)	91.9±13.9	91.9±13.6	0.983
UpH	6.0±0.8	6.2±2.6	0.201
PG (mg/dL)	129.0±48.0	119.4±19.2	0.734
HbA1c (%)	6.9±1.1	6.5±0.5	0.0042
SBP (mm Hg)	134.5±18.6	128.0±10.9	<0.0001
DBP (mm Hg)	80.1±10.3	76.4±7.2	<0.0001
Heart rate (bpm)	73.0±14.1	70.4±8.5	<0.0001
TC (mg/dL)	188.3±34.5	192.8±25.7	0.257
TG (mg/dL)	120.6±70.2	114.8±52.1	0.583
HDL-C (mg/dL)	55.7±15.8	57.5±15.3	0.019
BUN (mg/dL)	19.6±9.9	25.5±16.7	<0.0001
eGFR (mL/min/1.73 m <sup>2</sup> )	72.7±19.8	55.5±16.2	<0.0001
ACR (mg/g Cre)	24.8 (0.0–4735.6)	55.1 (3.8–4999.5)	<0.0001
UA (mg/dL)	6.2±1.7	6.5±1.4	0.046
ALT (U/L)	23.6±22.6	26.9±15.2	<0.0001
AST (U/L)	25.2±16.0	25.7±9.6	<0.0001
K <sup>+</sup> (mEq/L)	4.4±0.5	4.5±0.5	0.676
Na <sup>+</sup> (mEq/L)	140.8±2.3	139.9±2.6	0.0142
Cl <sup>-</sup> (mEq/L)	103.6±3.2	105.4±4.6	0.0002
TP (g/dL)	7.2±0.5	7.1±0.3	0.0018
Hb (g/dL)	12.8±1.7	13.1±1.3	0.0030
Plt (10 <sup>4</sup> /μL)	22.5±6.6	18.0±10.7	0.0003
WCC (10 <sup>3</sup> /μL)	6.2±1.8	6.1±1.5	0.241
PWV (cm/s)	1573.5±718.7	1612.3±423.2	0.283
ABI	1.1±0.1	1.1±0.2	0.211
IMT (mm)	0.7±0.3	1.3±1.1	<0.0001
8-OHdG (ng/mg Cre)	9.7±4.5	–	–

8-OHdG, 8-hydroxy-2'-deoxyguanosine; ABI, ankle brachial index; ACR, albumin–creatinine ratio; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; F, female; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; M, male; PG, plasma glucose concentration; Plt, platelet; PWV, pulse wave velocity; SBP, systolic blood pressure; TC, serum total cholesterol; TG, serum triglycerides; TP, total protein; UA, uric acid; UpH, urinary pH; WC, waist circumference; WCC, white cell count.

Table 1 shows the subjects' demographic and clinical data at the baseline and end point. Compared with the baseline, at the end point, a reduction was seen in the values of HbA1c (National Glycohemoglobin Standardization Program (NGSP) value), systolic BP (SBP), diastolic BP, heart rate, eGFR, sodium (Na<sup>+</sup>), serum high-density lipoprotein cholesterol (HDL-C), serum total protein, and platelets (Plt), while an increase was seen in the values of blood urea nitrogen (BUN), ACR, serum UA, alanine aminotransferase (ALT), aspartate transaminase (AST), chloride (Cl<sup>-</sup>), hemoglobin, and IMT. The values of BMI, WC, PWV, and ankle brachial index (ABI), etc, remained unchanged.

Table 2 shows the drugs that the participants were taking at the baseline and the end point. Compared with the baseline, the number of drug administrations increased at the end point for almost all treatment drugs, except sulfonylurea, with treatment having been reinforced. Regarding RAS inhibitors (RASIs), calcium

channel blockers, and diuretics, the number of concomitant therapies using two or more such drugs increased.

Table 3 shows the individual correlations between baseline UpH and each measurement item during the same period. A negative correlation was found between UpH and 8-OHdG, BMI, WC, UA, ACR, heart rate, BUN, ALT, AST, Cl<sup>-</sup>, Na<sup>+</sup>/Cl<sup>-</sup> ratio, white cell count (WCC), the number of RASIs taken, the number of drugs taken at the baseline, the number of drugs taken at the end point, and the changes in the number of drugs taken from the baseline to the end point. A positive correlation was seen between UpH and eGFR. We therefore conducted a multiple regression analysis, using UpH as the dependent variable and the factors that were correlated with UpH as the independent variables. This analysis found that the independent factors associated with UpH were 8-OHdG, UA, eGFR, ACR, heart rate, and the number of RASIs taken (see online supplementary table S1).

**Table 2** The drugs that the participants were taking at the baseline and end point

	Baseline		End point	
	N	(%)	N	(%)
<b>Antidiabetic agents</b>				
Insulin	158	(45.1)	196	(56.0)
Pioglitazone	63	(18.0)	103	(29.4)
Biguanide	147	(42.0)	207	(59.1)
DPP4I	–	(–)	83	(23.7)
Sulfonylurea	107	(30.6)	62	(17.7)
Glinide	9	(2.6)	92	(26.3)
AGI	68	(19.4)	133	(38.0)
<b>Antihypertensive agents</b>				
RASI therapy				
Mono	144	(41.1)	208	(59.4)
Double	123		131	
Triple	21		62	
Triple	0		15	
CCBs therapy				
Mono	71	(20.3)	174	(49.7)
Double	65		117	
Triple	6		45	
Triple	0		12	
Diuretics therapy				
Mono	37	(10.6)	68	(19.4)
Double	36		46	
Triple	1		18	
Triple	0		4	
β-blockers	17	(4.9)	42	(12.0)
Others	19	(5.4)	44	(12.6)
<b>Antihyperlipidemic agents</b>				
Statin	76	(21.7)	121	(34.6)

AGI, α-glucosidase inhibitors; CCBs, calcium channel blockers; DPP4I, dipeptidyl peptidase inhibitors; RASI, renin-angiotensin system inhibitors.

Table 4 shows the individual correlations between the %IMT, %PWV, %eGFR, Δlog ACR, and various parameters at baseline. The %IMT negatively correlated with UpH, eGFR, and HDL-C, and positively correlated with BMI, WC, UA, BUN, AST, ALT, rate of administration of RASIs, and the number of drugs taken. The %PWV had a weak negative correlation with UpH and eGFR and a positive correlation with ACR, BUN, and the number of drugs taken. The %eGFR correlated negatively with BMI, WC, UA, ACR, BUN, ALT, Cl<sup>-</sup>, Plt, WCC, the rate of RASI administration, and the number of drugs taken, and correlated positively with UpH, HDL-C, and the Na<sup>+</sup>/Cl<sup>-</sup> ratio. The Δlog ACR correlated negatively with UpH, eGFR, ACR, and the rate of RASI administration, and correlated positively with BMI, duration, SBP, and BUN.

In a multiple regression analysis that used the %IMT as the dependent variable and the factors that individually correlated with %IMT, described above, as the independent variables, only UpH was an independent risk factor (see online supplementary table S2A).

In a multiple regression analysis that used the %PWV as the dependent variable and the factors that individually correlated with %PWV, described above, as the independent variables, UpH and ACR were independent risk

**Table 3** The individual correlations between UpH and each measurement item at the baseline

Dependent variable=UpH	r	p Value
<i>Independent variable</i>		
8-OHdG	-0.60	<0.0001
Age	-0.08	0.86
BMI	-0.29	0.001
WC	-0.27	0.002
UA	-0.42	<0.0001
Gender	0.08	0.78
Duration	-0.10	0.75
Glucose	-0.11	0.32
HbA1c	-0.011	0.91
eGFR	0.300	<0.0001
ACR	-0.22	0.003
SBP	-0.16	0.08
DBP	-0.10	0.10
Heart rate	-0.22	0.008
TC	-0.00	0.91
TG	-0.12	0.09
HDL-C	0.15	0.07
BUN	-0.23	0.0001
ALT	-0.21	0.0003
AST	-0.18	0.0010
K <sup>+</sup>	-0.11	0.09
Cl <sup>-</sup>	-0.22	0.0002
Na <sup>+</sup>	0.04	0.87
Na <sup>+</sup> /Cl <sup>-</sup>	-0.26	<0.0001
Plt	-0.01	0.89
Hb	0.03	0.68
WCC	-0.23	0.0001
TP	-0.07	0.43
PWV	-0.03	0.54
ABI	0.01	0.90
IMT	0.09	0.35
RASIs	-0.21	0.0001
Drugs 2003	-0.52	<0.0001
Drugs 2013	-0.55	<0.0001
ΔDrugs	-0.36	<0.0001

ΔDrugs, changes in the number of drugs taken from the baseline to the end point; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; ABI, ankle brachial index; ACR, albumin-creatinine ratio; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; Drugs 2003, the number of drugs taken at the baseline; Drugs 2013, the number of drugs taken at the end point; eGFR, estimated glomerular filtration rate; Glucose, plasma glucose concentration; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; Plt, platelet; PWV, pulse wave velocity; RASIs, renin-angiotensin system inhibitors; SBP, systolic blood pressure; TC, serum total cholesterol; TG, serum triglyceride; TP, total protein; UA, uric acid; UpH, urinary pH; WC, waist circumference; WCC, white cell count.

factors (see online supplementary table S2B). In a multiple regression analysis that used the %eGFR as the dependent variable and the factors that individually correlated with %eGFR, described above, as the independent variables, UA, eGFR, ALT, and Plt were independent risk factors (see online supplementary table S2C). In a multiple regression analysis that used Δlog ACR as the

**Table 4** The individual correlations between the %IMT, %PWV, %eGFR,  $\Delta\log$  ACR and the measurements taken at the baseline

	IMT		PWV		eGFR		ACR	
	%IMT		%PWV		%eGFR		$\Delta\log$ ACR	
	r	p Value	r	p Value	r	p Value	r	p Value
UpH	-0.37	<0.01	-0.12	0.01	0.23	<0.01	-0.16	0.04
Age	0.03	0.71	0.00	0.98	0.08	0.05	-0.05	0.73
BMI	0.16	0.02	0.09	0.35	-0.17	0.05	0.13	0.05
WC	0.14	0.03	0.06	0.58	-0.14	0.05	0.06	0.26
UA	0.11	0.04	-0.06	0.57	-0.29	<0.01	-0.10	0.08
Gender	-0.08	0.30	0.06	0.42	0.05	0.16	0.03	0.76
Duration	-0.00	0.98	0.05	0.59	0.01	0.64	0.13	0.05
Glucose	0.03	0.73	-0.00	0.99	-0.01	0.79	0.06	0.67
HbA1c	0.01	0.95	-0.02	0.78	-0.12	0.08	-0.05	0.71
eGFR	-0.17	0.01	-0.10	0.04	-0.01	0.80	-0.15	0.04
ACR	0.02	0.59	0.31	<0.01	-0.26	<0.01	-0.28	<0.01
SBP	0.06	0.38	0.06	0.42	-0.07	0.12	0.16	0.04
DBP	0.04	0.61	0.02	0.77	-0.05	0.25	-0.09	0.43
Heart rate	0.09	0.26	-0.01	0.83	-0.02	0.58	0.04	0.74
TC	-0.08	0.28	-0.01	0.89	-0.03	0.74	-0.12	0.08
TG	0.04	0.63	-0.00	0.93	-0.17	0.06	-0.15	0.06
HDL-C	-0.11	0.04	-0.01	0.84	0.15	0.04	-0.00	0.98
BUN	0.11	0.04	0.12	0.04	-0.16	0.04	0.23	<0.01
ALT	0.12	0.03	0.08	0.51	-0.17	0.04	0.05	0.59
AST	0.15	0.01	-0.04	0.65	-0.08	0.18	0.01	0.92
K <sup>+</sup>	0.01	0.89	-0.08	0.49	-0.04	0.20	-0.07	0.43
Cl <sup>-</sup>	0.10	0.08	-0.05	0.53	-0.18	0.03	-0.05	0.57
Na <sup>+</sup> /Cl <sup>-</sup>	-0.10	0.07	-0.03	0.69	0.17	0.03	0.04	0.71
Plt	0.03	0.75	-0.01	0.80	-0.14	0.04	0.02	0.85
Hb	0.05	0.56	0.01	0.86	0.11	0.08	0.07	0.55
WCC	0.10	0.05	0.09	0.07	-0.15	0.04	-0.00	0.97
TP	0.07	0.11	0.09	0.21	0.10	0.26	0.13	0.06
RASIs	0.15	<0.01	-0.10	0.07	-0.14	<0.01	-0.14	<0.01
Total drugs	0.26	<0.01	0.17	<0.01	-0.23	<0.01	0.02	0.41
$\Delta$ Drugs	0.17	<0.01	0.14	0.01	-0.19	<0.01	-0.05	0.66

$\Delta$ Drugs, changes in the number of drugs taken from the baseline to the end point; ACR, albumin-creatinine ratio; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Glucose, plasma glucose concentration; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; Plt, platelet; PWV, pulse wave velocity; RASIs, renin-angiotensin system inhibitors; SBP, systolic blood pressure; TC, serum total cholesterol; TG, serum triglyceride; Total drugs, the number of drugs taken at the baseline; TP, total protein; UA, uric acid; UpH, urinary pH; WC, waist circumference; WCC, white cell count.

dependent variable and the factors that individually correlated with  $\Delta\log$  ACR, described above, as the independent variables, UpH, BMI, duration, ACR, and SBP were independent risk factors (see online supplementary table S2D).

In the logistic regression analysis, which used the occurrence of CKD (eGFR<60 mL/min/1.73 m<sup>2</sup>) as a dependent variable, UpH was an independent risk factor (p=0.001), with an OR of 0.522 777 (95% CI 0.3526216 to 0.77504). UpH was also an independent risk factor in our multiple regression analysis that used %eGFR as a dependent variable ( $\beta$ =2.365226, p=0.036).

Of the patients with UpH<6 (n=171), 28 had SAS, which was significantly more than that for the group of patients with UpH $\geq$ 6.0 (n=179), of whom 7 had SAS. Compared with the SAS (-) group (n=315), the SAS (+) group (n=35) had higher 8-OHdG, BMI, WC,  $\Delta\log$  ACR,

heart rate, serum triglyceride (TG), ALT, AST, Plt, WCC, %IMT, rate of increase in the number of RASIs administered, and number of drugs taken at the end point; they had a lower UpH, duration, %eGFR, HDL-C, and PWV (see online supplementary table S3). Of the SAS (+) group, those whose SAS was treated within the study period (SAS treatment (+), n=12) showed a greater rise in UpH than those whose SAS was not treated (SAS treatment (-), n=23) and a smaller increase in  $\Delta\log$  ACR and %IMT (see online supplementary table S4). However, since treatment was provided to all patients who were admitted to the hospital and underwent continuous positive airway pressure treatment, the BMI and WC values also decreased sharply in the treatment group. As a result, it is unclear if the changes seen in the SAS treatment (+) group were attributable to improvements in breathing conditions or weight reduction.

Among the participants in the UpH<6 group (n=171), 40 (23.4%) had high ALT values, which were more than those for the 17 participants (9.5%) in the UpH≥6 group (n=179). The number of cases of fatty liver in the UpH<6 group (n=36, 21.1%) was significantly greater than that in the UpH≥6 group (n=12, 6.7%).

## DISCUSSION

The patients enrolled in this study saw their BG, BP, and lipid levels remain under control or improve over the 10 years between 2003 and 2013. This was because a more rigorous control of these values became possible with continuing advancements in treatment drugs. In fact, the number of drugs administered to the patients in our study increased significantly from 2003 to 2013. However, no improvements were seen in terms of obesity, acidosis, fatty liver, hyperuricemia, and others, and the advancement of the renovascular disorders could not be completely halted. This indicates that merely improving BG, BP, and lipids cannot sufficiently suppress the advancement of renovascular disorders. Decreased UpH, in particular, was a powerful predictive factor for renovascular disorders. UpH is always determined as part of routine testing; it is a non-invasive and inexpensive test. The finding that UpH can predict long-term renovascular disorders has significant clinical value. The factors associated with UpH were OS, UA, eGFR, ACR, heart rate, and the number of RASIs being taken. This result concurs with the results of our previous study of individuals without diabetes.<sup>1</sup> The finding that a lower UpH is associated with a larger number of RASIs being taken appears to be because a lower UpH is associated with a greater number of patients with hypertension and increased ACR that was being treated effectively with RASIs. In (non-treated) participants without diabetes, acceleration of intrarenal RAS was closely related to lower UpH.<sup>1</sup> However, since RASIs powerfully suppress the acceleration of intrarenal OS and RAS,<sup>7</sup> urinary angiotensinogen excretion does not correlate with UpH in patients undergoing RASI treatment (data not shown). It appears that, in diabetic and obese participants, advanced glycation end-products (AGEs) such as methylglyoxal were formed, increasing the production of OS in the renal tubules and accelerating RAS, thereby reducing UpH.<sup>8–12</sup> Recently, however, the possibility is being suggested that low UpH may directly increase OS.<sup>5</sup>

We investigated the influence on the reduction of eGFR of the use of RASIs. The number of RASIs taken in 2003 (RASIs), and the number of RASIs additionally administered from 2003 to 2013 delta renin angiotensin system inhibitors (dRASIs) were not independent risk factors for the 10-year  $\Delta$  change in eGFR or %eGFR. (The RASIs in the  $\Delta$  change of eGFR:  $p=0.889$  and dRASIs:  $p=0.704$ ; and the RASIs in the %eGFR:  $p=0.938$  and dRASIs:  $p=0.454$ ). We attribute this to the large number of patients who were already taking RASIs at the

baseline stage, and the large number of long-term patients undergoing courses that had lasted from the period of having RASIs administered during observation to the period of evaluation.

A lowered UpH is caused by an increased supply of  $H^+$  to the urine or a reduction in the ability to eliminate  $H^+$ . An increased supply of  $H^+$  to the urine occurs either because of a reduction in blood pH (BpH) or the increased secretion of  $H^+$  from the renal tubules, while a reduction in the ability to eliminate  $H^+$  occurs as a result of a reduction in the supply of  $NH_3$  to the urine. BpH is defined according to the Henderson-Hasselbalch equation:  $BpH=7.62+\log(HCO_3^-/CO_2)$ . Therefore, a decline in BpH is believed to occur due to a reduced supply of  $HCO_3^-$  or an increased supply of  $CO_2$ . Since  $HCO_3^-$  is being supplied by the kidney and the liver, impaired hepatorenal function is believed to reduce BpH. Unfortunately, we did not measure urinary or serum bicarbonate levels in this study. It is unclear, therefore, whether or not UpH reflects blood acidity.

In our study also, patients with hepatorenal dysfunction showed a reduced UpH. Hepatorenal dysfunction is also closely related to obesity. In SAS, which is closely linked to obesity, the blood concentration of  $CO_2$  rises during the night, which is believed to cause a reduction in BpH. Hepatorenal disorders are a frequent complication of SAS, so a decrease in the supply of  $HCO_3^-$  appears to have been involved in the reduction of BpH. SAS was also strongly related to a decline in UpH and the advancement of renovascular disorders. Since treatment of SAS caused the value of UpH to rise and suppressed the advancement of renovascular disorders, SAS is most likely one cause of UpH reduction and advancement of renovascular damage. SAS accelerates sympathetic nerve activity, inducing hypertension and an increased heart rate.<sup>13</sup>

One cause of the increased secretion of  $H^+$  in the renal tubules is the activation of the  $Na^+-H^+$  exchanger (NHE) that is expressed in the renal proximal tubules.<sup>14–15</sup> In diabetes and obesity, the NHE is activated by the suppression of megalin expression due to increases in OS or RAS activity.<sup>5, 12, 16, 17</sup> Increased OS inside the renal tubule cells increases the level of transforming growth factor  $\beta$ , which suppresses the expression of megalin.<sup>1, 18–21</sup> The reduction in megalin results in expanded NHE activity, thereby increasing the secretion of  $H^+$  (see online supplementary figure S1).<sup>22</sup> The ability to eliminate  $H^+$  is controlled by the secretion of  $NH_3$  into the urine.<sup>23</sup> Nitrogen (N) needed for synthesizing  $NH_3$  is supplied by glutamine.<sup>24</sup> If OS increases, NF-E2-related factor 2 (Nrf<sub>2</sub>), which is an anti-OS transcription factor, is activated. Since Nrf<sub>2</sub> promotes the metabolism of glutamine to lactate, there is a possibility that the supply of N from glutamine may decrease and the production of  $NH_3$  may decline<sup>25</sup> (see online supplementary figure S2). The amount of  $NH_3$  is reported to be lower in patients with diabetes.<sup>3</sup> A high-protein diet increases BUN,<sup>26</sup> decreases the secretion of  $NH_3$

into the urine, and reduces UpH as a result.<sup>27</sup> The content of the patient's meal might be a cause of lower UpH.

Lower UpH was closely related to higher UA values. Conditions that elevate serum UA concentration are closely related to decreases in UpH. Under IR conditions, the serum UA concentration rises because of the increased production of UA in the liver or adipose tissues and decreased renal excretion of UA (see online supplementary figure S2).<sup>25–28</sup> The UA production is increased by AMP deaminase activation induced by fructokinase activated by hyperglycemia (see online supplementary figure S3). This pathology is closely involved with the advancement of renal damage.<sup>29</sup>

Hyperinsulinemia increases the reabsorption of UA.<sup>30</sup> There is a possibility that an OS-induced increase in lactate is involved in this reabsorption increase.<sup>25</sup>

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